Effects of Submicrometer Particle Compositions on Cytokine Production and Lipid Peroxidation of Human Bronchial Epithelial Cells

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To identify the size and components related to toxicity of ambient particles, we used a trichotomous impactor to collect 17 sets of particles in three size ranges—submicrometer (diameters < 1 μm; $PM_{1.0}$), fine (diameters between 1 and 2.5 μm; $PM_{1.0–2.5}$), and coarse (diameters between 2.5 and 10 μm; $PM_{2.5–10}$)—at stations monitoring background, urban, traffic, and industrial air in Taiwan. Elemental contents, carbon contents, soluble ions, and endotoxin content of particles were determined by X-ray fluorescence spectrometry, thermal analysis, ion chromatography, and the Limulus amebocyte lysate assay, respectively. Human bronchial epithelial BEAS-2B cells were exposed to particle extracts at 100 μg/mL for 8 hr, and interleukin-8 (IL-8) concentrations in the medium and lipid peroxidation products were measured. Particle-induced tumor necrosis factor-α (TNF-α) production by mouse macrophage RAW 264.7 cells was also measured. $PM_{1.0}$ stimulation resulted in significantly higher IL-8 production and lipid peroxidation than $PM_{2.5–10}$, whereas the responses elicited by $PM_{1.0–2.5}$ were not significantly higher than blank filters. Untreated and polymyxin B–pretreated $PM_{1.0}$ also stimulated more TNF-α production by RAW 264.7 cells than $PM_{2.5–10}$ and $PM_{1.0–2.5}$. Cytokine production was significantly associated with metal contents of $PM_{1.0}$: IL-8 correlated with Cr and Mn, and TNF-α correlated with Fe and Cr. Lipid peroxidation in BEAS-2B cells correlated with elemental and organic carbon contents. Our study found that size and composition of ambient particles were both important factors in inducing cytokine production and lipid peroxidation. *Key words:* cytokine, human bronchial epithelial cell, lipid peroxidation, macrophage, submicrometer particle. *Environ Health Perspect* 111:478–482 (2003). doi:10.1289/ehp.5519 available via *http://dx.doi.org/* [Online 1 November 2002]

Epidemiologic studies have demonstrated increases in cardiovascular and respiratory morbidity and mortality in association with elevated mass concentration of ambient particulate matter, especially that of fine particles (Dockery and Pope 1996). Because respiratory epithelium and macrophages are the cells that come in direct contact with inhaled particles, *in vitro* experiments have often used these two cell types to study the toxicity of particles, including studies addressing concerns about the effects of particle size and specific particle components.

Fine particles contain various combustion products, including transition metals and acids, and are better associated with health effects than are coarse particles. However, this has not been consistently reflected in *in vitro* studies. In fact, in experiments where macrophages or monocytes were used, coarse particles sometimes caused greater cellular responses than fine particles. This could in part be attributed to the sensitivity of macrophages to endotoxin, which was more abundant in coarse particles (Huang et al. 2002; Monn and Becker 1999; Soukup and Becker 2001). Respiratory epithelium possesses the ability to respond to diesel exhaust particles (DEP), coal fly ash, and cigarette smoke particles. Alveolar epithelium A549 cells were found to bind particles through scavenger receptors, and alpha-quartz particles stimulated these cells to produce interleukin 8 (IL-8) (Stringer et al. 1996). Iron-containing coal fly ash stimulated A549 cells to produce IL-8, and the response was most remarkable when the particles were enriched in submicrometer particles (diameters < 1 μm; $PM_{1.0}$) compared with larger size fractions [diameters of 2.5 μm ($PM_{2.5}$) or between 2.5 and 10 μm ($PM_{2.5–10}$)] (Smith et al. 2000).

Particle toxicity may be related to the contents of transition metals, organic compounds, biologic compounds, and acidic secondary pollutants (nitrate and sulfate). Transition metals may enhance intracellular production of oxidants, with ensuing cell activation or injury. Metal-containing residual oil fly ash, coal fly ash, and some transition metals were found to result in enhanced expression of proinflammatory cytokines in cell culture systems (Broeckaert et al. 1999; Dye et al. 1999; Samet et al. 1998; Smith et al. 2000). However, there has been little direct evidence correlating cellular responses with ambient particle components. In one such investigation, particle-induced oxidant generation in polymorphonuclear leukocytes was related to insoluble Si, Fe, Mn, Ti, and Co content of particles but not to soluble transition metals (Prahalad et al. 1999). Regarding other particle components, except for the well-recognized effect of bacterial endotoxin, evidence for the importance of other organic components such as polycyclic hydrocarbons has only begun to accumulate (Bonvallot et al. 2001). To examine the size and component effects of ambient particulates, particle samples in three size ranges, $PM_{1.0}$, $PM_{1.0–2.5}$ (diameters between 1 and 2.5 μm), and $PM_{2.5–10}$, were extensively characterized and correlated with cytokine-inducing and oxidative stress–inducing bioactivities in respiratory epithelial cells. We also examined whether cytokine production in RAW 264.7 cells could be affected by different sizes and components of ambient particulates. Considering the sensitivity of macrophages to bacterial endotoxin, we performed assays for particles with and without polymyxin B pretreatment.

Methods

Particle collection. We collected ambient particles at four ambient air monitoring stations of the Taiwan Air Quality Monitoring Network, which were representative of background, urban, traffic, and industrial air pollution patterns. We used a trichotomous particle sampler (Particle Technology Laboratory, MN, USA) with a flow rate of 40 ft^3/min to collect submicrometer ($PM_{1.0}$), fine ($PM_{1.0–2.5}$), and coarse ($PM_{2.5–10}$) ambient particles. We collected a total of 17 sets of ambient air samples between September and December of 2000. Each set included $PM_{1.0}$ on two 47-mm Teflon filters and one 8 inches × 10 inches quartz filter, $PM_{1.0–2.5}$ on two 47-mm Teflon filters, and $PM_{2.5–10}$ on one 47-mm Teflon and one 2.5 inches × 7 inches quartz filter. The sampling duration lasted for 8–37 hr, depending on local particle concentrations.

X-ray fluorescence analysis. Particles on Teflon filters were examined by an energy-dispersive X-ray fluorescence system (model Ex6600AF, Jordan Valley Applied Radiation, Austin, TX, USA) to determine the contents of 26 elements: Na, Mg, Al, Si, P, S, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn,

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